Title: Genetic and neutralization properties of the envelope gene in HIV-1 and HIV-2 monotypic and dual infections.

I. Concept:

HIV-2 reports a lower pathogenecity than HIV-1 and HIV-2 sera depict a significant capacity to neutralize autologous and heterologous viruses, indicating that neutralization escape is rare in HIV-2 infection (Ozkaya S. G *et al*, 2012, Esbjörnsson J. *et al* 2012). In case of HIV-1+2 (dual) infections there exists a lack of information on genetic, functional characteristics of the envelope gene and the neutralizing antibody responses. In countries where mono infections and dual infections of HIV-1 and HIV-2 are present, it is essential to draw comparison between all the three infection patterns. Since the host cell entry through the envelope appears to be the dominant factor controlling HIV fitness and neutralizing (nAb) responses, we propose to study the genetic diversity of the envelope gene and variability in the nAb responses in HIV-1, HIV-2 and HIV-1 and 2 dually infected individuals.

Determining the factors causing the rare escape to neutralization in case of HIV-2 that control viremia, factors in HIV-1 infection that complicate its neutralization and elucidating the nAb pattern in dual infections will generate a comparative account of these differences. Correlation of the neutralization data and envelope genetic diversity will help in understanding the viral dynamics that provide favorable conditions for the host to mount a better immune control over the virus in case of HIV-2 infection. The results from the study may provide useful insight for designing therapeutic modalities and point a way for designing vaccine strategies.

II. Hypothesis:

1. In HIV-1, HIV-2 and HIV-1 and 2 dual infections the viral envelope gene undergoes genetic diversity keeping intact some nAb eliciting antigenic signatures.
2. The pattern within the antigenic signatures of the envelope gene impacts the characteristic neutralization responses in the monotypic and dual HIV-1 and HIV-2 infections.

III. Objectives:

1. To characterize *env* gene of viruses isolated from HIV-1 and HIV-2 monotypic and dually infected patients in order to study the genetic diversity.

2. To study autologous, heterologous and cross neutralization patterns in HIV-1 and HIV-2 monotypic and dually infected serum samples.

Approval Valid up to
31 JUL 2013
02. Nov. '12
Dr. V. S. PADBIDRI
Chairman, Ethics Committee
National AIDS Research Institute, Pune.
02 NOV 2012
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IV. Methodology:

Study samples: 40ml of whole blood will be collected from (Min 5 and Max10) HIV-1, HIV-2 and HIV-1 and 2 (dual infected) patients at three different time points (0, 6 and 12 months) after obtaining an informed consent.

Objective 1:

Viruses from HIV-1, HIV-2 and HIV- 1 and 2 dually infected samples will be isolated using co-cultivation method. DNA will be extracted from cultured PBMC and the env gene will be amplified by PCR using various sets of primers. The env PCR products will be cloned in suitable mammalian expression vector and sequencing will be performed. Individual *env* sequence fragments for each clone will be assembled and edited using the Seqscape software. DNA & Protein sequences generated will be aligned using CLUSTAL X, MEGA 4.1 softwares and molecular phylogenetic and nAb signatures will be studied.

Objective 2:

Functional envelope clones will be generated and will be cotransfected with an envelope deficient backbone plasmid in 293T cells to generate *env* pseudoviruses. After determination of the TCID50, the neutralization assays will be performed by using different checker board titrations for autologous, heterologous and cross neutralization in the standard TZM-bl assay along with standard reference strains and MAbs. The results obtained from the wet lab experiments will be compared and analyzed using various in silico tools/softwares for determining nAb patterns and epitope prediction.

V. Possible Outcomes of the study:

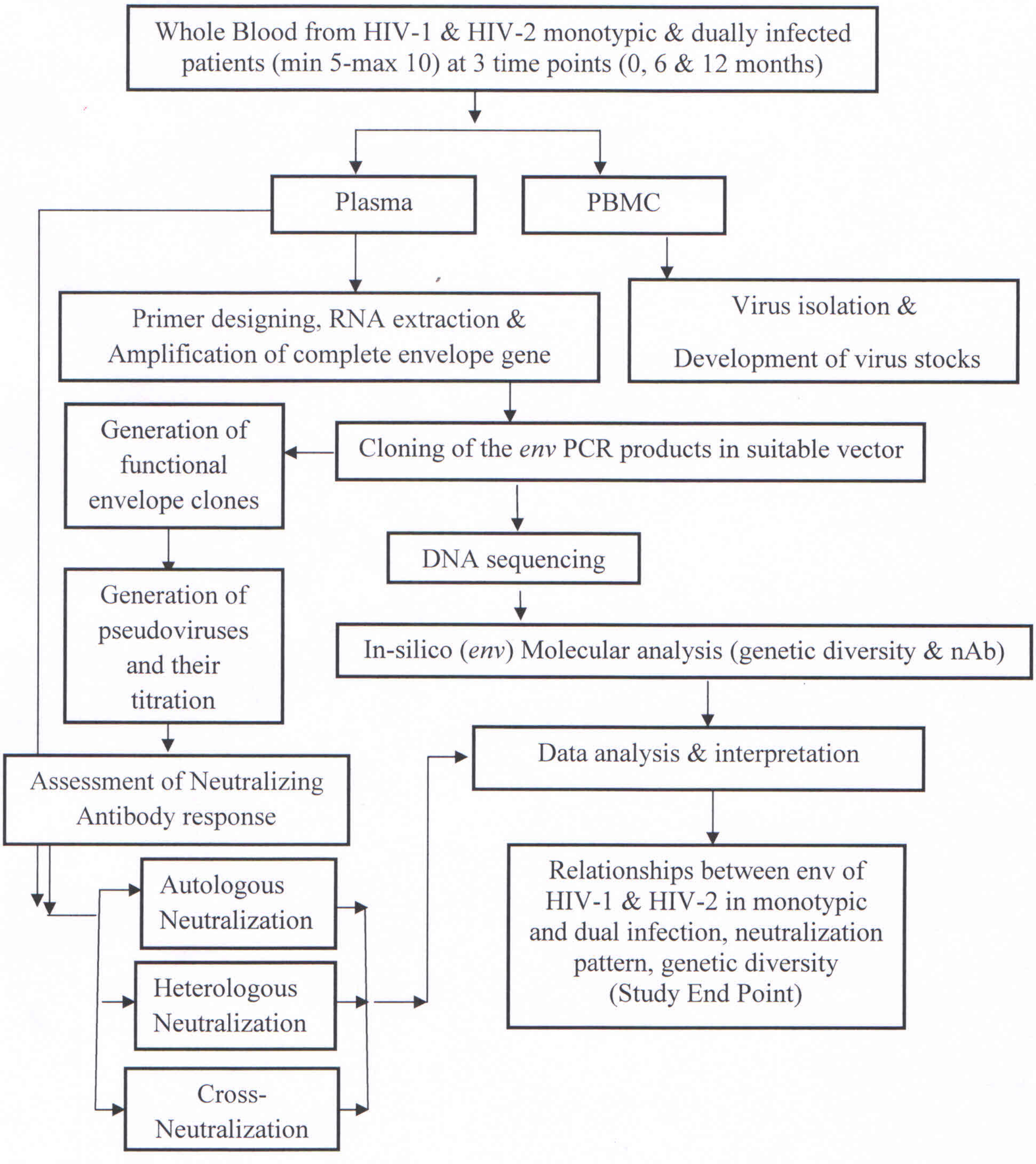
The molecular data generated from the study would provide important highlights of the genetic characteristics of the envelope gene in HIV-1 and 2 monotypic and dual infections.

The study will generate a comparative information on neutralization patterns, nAb epitopes that will provide key insights for therapeutic and vaccine research.

VI. Budget:

The overall budget for the project is **Rs. 15, 20,000/-**. This includes purchase of chemicals reagents, kits and consumables etc.

VII. Study Overview:


Whole Blood from HIV-1 & HIV-2 monotypic & dually infected patients (min 5-max 10) at 3 time points (0, 6 & 12 months)
Plasma
PBMC
Primer designing, RNA extraction & Amplification of complete envelope gene
Virus isolation & Development of virus stocks
Generation of functional envelope clones
Cloning of the *env* PCR products in suitable vector
DNA sequencing
Generation of pseudoviruses and their titration
In-silico (*env*) Molecular analysis (genetic diversity & nAb)
Assessment of Neutralizing Antibody response
Data analysis & interpretation
Autologous Neutralization
Heterologous Neutralization
Cross-Neutralization
Relationships between env of HIV-1 & HIV-2 in monotypic and dual infection, neutralization pattern, genetic diversity (Study End Point)

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